DELIVERY OF IMMUNE RESPONSE MODIFIER COMPOUNDS USING METAL-CONTAINING PARTICULATE SUPPORT MATERIALS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a Continuation-In-Part of U.S. Patent Application Serial No. 10/640904, filed on August 14, 2003, and claims priority to U.S. Provisional Patent Application Serial Nos. 60/462140, filed on April 10, 2003, 60/545424, filed on February 18, 2004, 60/515256, filed on October 29, 2003, and 60/545542, filed on February 18, 2004, each of which is incorporated herein by reference in their entirety.

BACKGROUND

There has been a major effort in recent years, with significant successes, to discover new drug compounds that act by stimulating certain key aspects of the immune system, as well as by suppressing certain other aspects (see, e.g., U.S. Pat. Nos. 6,039,969 and 6,200,592). These compounds, referred to as immune response modifiers (IRMs), appear to act through basic immune system mechanisms known as toll-like receptors to induce selected cytokine biosynthesis and may be used to treat a wide variety of diseases and conditions. For example, certain IRMs may be useful for treating viral diseases (e.g., human papilloma virus, hepatitis, herpes), neoplasias (e.g., basal cell carcinoma, squamous cell carcinoma, actinic keratosis), and T_H2-mediated diseases (e.g., asthma, allergic rhinitis, atopic dermatitis), and are also useful as vaccine adjuvants. Many of the IRM compounds are small organic molecule imidazoquinoline amine derivatives (see, e.g., U.S. Pat. No. 4,689,338), but a number of other compound classes are known as well (see, e.g., U.S. Pat. No. 5,446,153) and more are still being discovered. Other IRMs have higher molecular weights, such as oligonucleotides, including CpGs (see, e.g., U.S. Pat. No. 6,194,388. In view of the great therapeutic potential for IRMs, and despite the important work that has already been done, there is a substantial ongoing need for new means of controlling the delivery and activity of IRMs in order to expand their uses and therapeutic benefits.

SUMMARY

It has now surprisingly been found that immune response modifiers (IRMs) of the invention can be attached to support materials that include a metal and, importantly, that they retain biological activity even while they remain attached to such material. This ability to attach IRMs to metal-containing supports, such as gold particles, and to form biologically active IRM-support complexes allows for a tremendous range of useful applications. For example, where one may wish to use metal-containing complexes to deliver the IRMs, such as gold particles used in certain needless injection devices, and/or where one may not wish to release all the IRM compound to be effective.

That is, in contrast to eluting drug from a coated surface or delivering drug from a formulation, the IRMs here can be active while attached to particulate support materials that include a metal. This approach can be used, e.g., to help reduce systemic absorption through dermal, mucosal and other tissues, as well as to maintain extended deposition of the IRM at an intended site of action, such as implanted in a solid tumor mass.

Moreover, not only has it been found that the IRMs are still biologically active when attached to a support complex, but surprisingly, the cytokine induction profile of the IRM can be altered in potentially desirable ways by virtue of such attachment.

The IRM may be covalently or non-covalently bound, preferably covalently bound, to the particulate support material. Attachment of an IRM to a particulate support material provides for the localized biological activity of the IRM and typically prevents, or at least reduces the occurrence of, the systemic distribution of the IRM.

Accordingly, the present invention provides an IRM-support complex that includes at least one IRM compound attached to particulate support material including at least one metal. In some embodiments, the IRM compound is covalently attached to the support material that includes the metal. Typically, the IRM compound is covalently attached to at least one of the metals.

In some embodiments, the support material is in the form of porous particles or solid particles. The solid particles are typically in the form of solid metal particles.

In certain embodiments of the present invention, the support material is coated with one or more metals or alloys thereof. In other embodiments, the metal forms the core of the support material and is coated with another material, which may be an organic polymer, for example.

In certain embodiments, the particles may be solid metal particles. In other embodiments, the support material includes an organic polymer or an inorganic polymer, the latter of which is typically in the form of a metal oxide, such as a glass or a ceramic.

In certain embodiments of the present invention, the particulate support material (including one or more metals) has an average density of 0.1 grams per cubic centimeter (g/cm^3) to 25 g/cm^3. For certain applications, the particulate support material has an average density of 5 g/cm^3 to 20 g/cm^3 (preferably, 10 g/cm^3 to 20 g/cm^3).

In certain embodiments, the particulate support material (including one or more metals) has an average particle size of 1 nanometer (nm) to 250 microns (micrometers, μm). For certain applications, the particulate support material has an average particle size of 5 nm to 100 nm. For certain applications, the particulate support material has an average particle size of 10 nm to 50 microns. For certain applications, the particulate support material has an average particle size of 0.1 micron to 20 microns. For certain applications, the particulate support material has an average particle size of 0.2 micron to 5 microns.

The metal is typically a transition metal, preferably selected from the group consisting of Groups 6-11 of the Periodic Table, and more preferably selected from the group consisting of tungsten, iron, gold, silver, platinum, nickel, cobalt, rhodium, zirconium, titanium, and combinations thereof. For certain embodiments, silicon-based materials (e.g., silica-based materials can be used). Thus, herein, the term "metal" includes metalloids such as silicon. Alternatively, rare earth elements (i.e., the lanthanides and actinides) can be used. The metal can be in the form of an alloy or a complex (e.g., a metal-organic complex or a metal oxide), for example.

In certain embodiments, the particulate support material has magnetic properties, either permanent magnetic, paramagnetic, or superparagmetic, preferably, superparamagnetic. The particulate support material for such embodiments preferably include iron, nickel, cobalt, tungsten, titanium, rare earth elements, or combinations thereof. For such embodiments, the IRM-support complex can be guided into the host, relocated, redistributed inside the host, and/or removed from the host by an external magnetic field. The particulate support material for such embodiments can also be used to enhance the capacity of radiological diagnostics such as in magnetic resonance imaging.

In some embodiments, the IRM-support complex may further include an additional drug. The IRM compound and the additional drug may be coated onto at least a portion of the particulate support material. The additional drug may be a vaccine, including, for example, a DNA vaccine. The IRM compound may be physically or chemically linked to the vaccine so as to form a unit. The additional drug may be linked directly to the particulate support material separately from the directly linked IRM.

In some embodiments of the present invention, the IRM compound may be an agonist of at least one TLR, preferably an agonist of TLR6, TLR7, or TLR8. The IRM may also in some cases be an agonist of TLR 9. In some embodiments of the present invention, the IRM compound may be a small molecule immune response modifier (e.g., molecular weight of less than about 1000 daltons).

In some embodiments of the present invention, the IRM compound may comprise a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring, or a 4-aminopyrimidine fused to a five membered nitrogen-containing heterocyclic ring.

In some embodiments of the present invention, at least one IRM compound may be an imidazoquinoline amine such as, for example, an amide substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amine, or a 6-, 7-, 8-, or 9-aryl or heteroaryl substituted imidazoquinoline amine; a tetrahydroimidazoquinoline amine such as, for example, an amide substituted tetrahydroimidazoquinoline amine, a sulfonamide substituted tetrahydroimidazoquinoline amine, a urea substituted tetrahydroimidazoquinoline amine, a aryl ether substituted tetrahydroimidazoquinoline amine, a heterocyclic ether substituted tetrahydroimidazoquinoline amine, an amido ether substituted tetrahydroimidazoquinoline amine, a sulfonamido ether substituted tetrahydroimidazoquinoline amine, a urea substituted tetrahydroimidazoquinoline ether, or a thioether substituted tetrahydroimidazoquinoline amine; an imidazopyridine amine such as, for example, an amide substituted imidazopyridine amine, a sulfonamide substituted imidazopyridine amine, a urea substituted imidazopyridine amine, an aryl ether substituted imidazopyridine amine, a heterocyclic ether substituted imidazopyridine amine, an amido

ether substituted imidazopyridine amine, a sulfonamido ether substituted imidazopyridine amine, a urea substituted imidazopyridine ether, or a thioether substituted imidazopyridine amine; a 1,2-bridged imidazoquinoline amine; a 6,7-fused cycloalkylimidazopyridine amine; an imidazonaphthyridine amine; a tetrahydroimidazonaphthyridine amine; an oxazoloquinoline amine; a thiazoloquinoline amine; an oxazolopyridine amine; a thiazolopyridine amine; an oxazolonaphthyridine amine; a thiazolonaphthyridine amine; a 1*H*-imidazo dimer fused to a pyridine amine, a quinoline amine, a tetrahydroquinoline amine, a naphthyridine amine, or a tetrahydronaphthyridine amine; pharmaceutically acceptable salts thereof; and combinations thereof.

In some embodiments, at least one IRM compound may be a purine, imidazoquinoline amide, benzimidazole, 1*H*-imidazopyridine, adenine, or a derivative thereof.

In certain embodiments, the present invention provides an IRM-support complex that includes at least one IRM compound covalently attached to particulate support material including at least one zero-valent transition metal, wherein the particulate support material has an average density of 10 g/cm^3 to 20 g/cm^3.

The IRM-support complex may be contained in a delivery device, such as a so-called gene gun or needle-free injection system. The IRM-support complex can be delivered by ballistic force or magnetic acceleration, for example. Thus, in one aspect of the invention there is provided a delivery device that includes a reservoir containing an IRM-support complex comprising at least one IRM compound on particulate support material comprising at least one metal. After delivery, e.g., from a DNA vaccine gene gun or other needle-free injection system, the IRM may be active while remaining attached and/or may be active after detachment from the support complex. Also, particles used in such devices may have both an IRM and vaccine, e.g., DNA or other vaccine, attached to the same particles, or the IRM and vaccine may be separated, for example each on separate particles.

In certain embodiments, the present invention provides an IRM-support complex that includes at least one IRM compound covalently attached to particulate support material including at least one zero-valent transition metal, wherein the particulate support material has an average particle size of 0.2 micron to 5 microns. Such IRM-support complexes are particularly desirable for deposition of an IRM into the lungs of a subject.

Such IRM-support complexes are also desirable for deposition in solid tumors following intravenous administration due to the increased tumor capillary permeability. Particles useful for targeting delivery to tumors can have an average particle size of 5 nm to 100 nm.

In certain embodiments, the present invention provides an IRM-support complex that includes at least one IRM compound covalently attached to particulate support material including at least one zero-valent transition metal selected from the group consisting of Groups 6-11 of the Periodic Table. Such IRM-support complexes are particularly desirable for visualization of the location of an IRM. In certain embodiments, the signal (e.g., a magnetic resonance signal) from the IRM-support complex can be recorded to generate 2- or 3-dimensional images and used as diagnostics for the host.

In certain embodiments, the present invention provides an IRM-support complex that includes at least one IRM compound covalently attached to a tether, such as an oligonucleotide, which attaches by physical attraction (e.g., static forces, hydrogen bonding, hydrophobic-hydrophilic interactions) to the particulate support material. Preferably, for this embodiment, the particulate support material has an average particle size of 2 microns to 5 microns.

The present invention also provides methods of delivering an IRM to a subject that includes delivering an IRM-support complex of the present invention. Delivery devices having a reservoir that includes one or more of the IRM-support complexes of the present invention is also provided.

A method of making an IRM-support complex is also provided, wherein the method includes attaching an immune response modifier to a particulate support material that includes at least one metal. Preferably, the method of attaching includes covalently attaching the IRM. This can occur by modifying the IRM to include an alkoxysilane moiety. The IRM-modified alkoxysilane is attached to a silicon-containing particulate support material, which can include silica particles.

The term "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims,

As used herein, "a," "an," "the," "at least one," and "one or more" are used interchangeably. Thus, for example, an IRM-support complex comprising "an" IRM compound can be interpreted to mean that the complex includes at least one IRM

compound. Similarly, for example, particulate support material comprising "a" metal can be interpreted to mean that the particulate support material includes at least one metal.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used individually and in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS OF THE INVENTION

The present invention is directed to the attachment of cytokine inducing and/or suppressing immune response modifiers (IRMs) to particulate support materials that include a metal to form IRM-support complexes. The IRMs retain biological activity following such attachment to a particulate support material. IRM-support complexes allow for the localized delivery of an IRM to a desired location in the body of a subject and typically prevent, or at least reduce the occurrence of, the systemic distribution of the IRM.

Significant advantages can be realized from the present invention. For example, the metal-containing IRM-support complex can be used with a delivery device, such as a gene gun, for delivery of the IRM. The metal of the metal-containing IRM-support material can be used for visualization of the location of deposition of the IRM-support complex. The metal of the metal-containing IRM-support complex can be used for absorption of energy from an external energy source (e.g., microwave, x-ray, UV light) to break the linkage with the IRM.

As used herein, "particulate support material" is a particulate material (i.e., material in the form of particles) that is itself generally biologically inactive. As used herein, "generally biologically inactive" means that cellular interaction with the material does not appreciably alter the phenotype of the cell. The particulate support material may

be of a size and chemical nature to prevent the engulfment or penetration of the particulate material into cells, in which case the IRM-support complex retains an extracellular location. Alternatively, the macromolecular support material may be of a size and chemical nature to allow engulfment by cells. For example, the macromolecular support material may be of a size and chemical nature to allow selective deposition in solid tumors on the basis of the tumor's increased vascular permeability. The terms "substrate," "support material," or "support," may also be used herein to refer to a particulate support material that includes a metal, an alloy, or a metal complex.

Typically, the metal-containing particulate support material is in the form of porous or solid particles. The solid particles are typically in the form of solid metal-containing particles, which may be zero-valent metal particles (e.g., gold particles).

The support material can be coated or impregnated with one or more metals. Alternatively, the support material can include one or more metals as the core. Alternatively, the support material can be in the form of metallic particles (e.g., gold particles), which may be porous or solid.

The support material can include an organic polymer or an inorganic polymer, the latter of which is typically in the form of a metal oxide, which can be in the form of a glass or a ceramic. If the support includes an organic polymer it also includes a metal, which can be a zero-valent metal. If the support includes an inorganic polymer, there may be no need for an additional metal. Alternatively, a different material containing a metal, such as a zero-valent metal (although other oxidations states of the metal are also possible), may be included in the support material. Other inorganic and/or organic materials can be used as the support material as long as it includes a metal, in any of a variety of oxidation states.

The particulate support material can possess a wide range of densities. For certain embodiments, the particles have an average density of at least 0.1 gram per cubic centimeter (g/cm^3), and for certain embodiments at least 5 g/cm^3, and for certain embodiments at least 10 g/cm^3. For certain applications, the particles have an average density of no greater than 25 g/cm^3, and for certain embodiments at no greater than 20 g/cm^3. These values of densities are for the particulate support material that includes one or more metals.

The particulate support material can possess a wide range of particle shapes and sizes. Herein, the average particle size is the average of the longest dimension of the particles. The particles are preferably spherical and the average particle size is the average diameter. The particles preferably have an average particle size of at least 1 nanometer (nm), although in certain situations it may even be as low as 0.1 nm. For certain embodiments the average particle size is at least 2 nanometers; for certain embodiments it is at least 5 nm; for certain embodiments it is at least 10 nm; for certain embodiments it is at least 0.1 micron; for certain embodiments it is at least 0.2 micron; and for certain embodiments it is at least 2 microns. For certain embodiments the average particle size is no greater than 250 microns; for certain embodiments it is no greater than 50 microns; for other embodiments it is no greater than 20 microns; for other embodiments it is no greater than 10 microns; for certain other embodiments the average particle size is no greater than 5 microns; for certain embodiments it is no greater than 100 nm; for other embodiments it is no greater than 10 nm; and for other embodiments it is no greater than 5 nm. These values of particle sizes are for the particulate support material that includes one or more metals, which can be in the form of zero-valent metal or in the form of a metal-containing compound having a non-zero valency, for example.

The metal can possess a wide range of electron densities, depending on the desired application. The metal is typically a transition metal, preferably selected from the group consisting of Groups 6-11 of the Periodic Table, and more preferably selected from the group consisting of tungsten, iron, gold, silver, platinum, nickel, cobalt, rhodium, zirconium, titanium, and combinations thereof. For certain embodiments, silicon-based materials (e.g., silica-based materials can be used). Thus, herein, the term "metal" includes metalloids such as silicon. Alternatively, rare earth elements (i.e., the lanthanides and actinides) can be used as the metal. The metal can be in the form of an alloy or a complex (e.g., a metal-organic complex or a metal oxide), for example. Thus, herein, the term "metal" includes metalloids such as silicon in addition to transition metals, main group metals, rare earth metals, which my or may not be in their zero-valent state.

In certain embodiments, the particulate support material has magnetic properties, either permanent magnetic, paramagnetic, or supermagnetic, preferably, superparamagnetic. The particulate support material for such embodiments preferably include iron, nickel, cobalt, tungsten, titanium, rare earth elements, or combinations

thereof. The particulate support material for such embodiments can also be used to enhance the capacity of radiological diagnostics such as in magnetic resonance imaging.

In an IRM-support complex, preferably an IRM is attached to a particulate support material. This attachment may be directly to the metal incorporated in the particulate support material. As used herein, the term "attached" includes both covalent bonding and non-covalent chemical association (e.g., ionic bonding, hydrophobic bonding, and hydrogen bonding) of an immune response modifier with a particulate support material. Preferably, the immune response modifiers are attached to a particulate support material by means of covalent bonding and hydrogen bonding. Preferably, this attachment is to the metal present in the particulate support material. The terms "coupled," "conjugated," "bonded," or "immobilized" may also be used herein to represent "attached."

The IRM is coated on, impregnated within, or attached to the support material by a sufficiently strong bond (which sometimes may require a covalent bond) so that under the circumstances of intended use the IRM is biologically active during use while it is attached to the support. It should also be understood that for each of the uses described herein an IRM may be provided in an unattached, releasable form, or become unattached over time, so that the IRM can be released and function in that manner. Mixtures of the two types can also be used where desirable.

The IRM-support complex of the present invention provides for the localized biological activity of the IRM. Preferably, the IRM is attached to the particulate support material. For example, the IRM can be attached as a side group to a polymer, and the polymer coated onto a metal core. In certain embodiments, the present invention provides an IRM-support complex that includes at least one IRM compound covalently attached to a tether, such as an oligonucleotide, or an antibody, or an antigen, which couples by physical attraction (e.g., static forces, hydrogen bonding, hydrophobic-hydrophilic interactions) to the particulate support material.

Although the IRM may eventually detach from the particulate support material (e.g., through biodegradation of a polymer to which the IRM is attached, for example), the IRM preferably does not detach during a suitable period of use while it is active (although it may of course also be active after detachment). Such attachment of an IRM to a particulate support material can be used to reduce the occurrence of, or prevent, the systemic absorption of the IRM, and can minimize the systemic side effects sometimes

observed with the systemic administration of an IRM. Also, such attachment of an IRM to a substrate can serve to limit or focus the effect of the IRM to a localized region for a desired duration, and if the support material can be removed, the IRM can then be easily removed at will along with it. This provides very important control over where and how long the IRM is applied.

The substrate having the IRM attached thereto can be used in a variety of medical applications, which can be therapeutic, prophylactic (e.g., as a vaccine adjuvant), or diagnostic. As used herein, "treating" a condition or a subject includes therapeutic, prophylactic, and diagnostic treatments.

In some embodiments, an IRM-support complex of the present invention can be used in, e.g., ex-vivo treatment of immune cells, experimental testing, or a diagnostic assay in which an IRM is a component. For example, use of an IRM-support complex can enhance cellular contact with an IRM, can facilitate the removal of an IRM from a diagnostic assay, can allow for the concentrated delivery of an IRM, and can assist in the conservation of IRM reagents.

In addition to one or more IRM compounds, the IRM-support complexes and methods of the present invention can include additional agents administered in a composition with the IRM-support complexes. Alternatively, the additional agent(s) can be administered separately from the IRM-support complexes. Such additional agents may be additional drugs, including, for example, a vaccine, a tumor necrosis factor (TNF) agonist, or a tumor necrosis factor receptor (TNFR) agonist. Vaccines include any material that raises either humoral and/or cell mediated immune response, such as live or attenuated viral and bacterial immunogens and inactivated viral, tumor-derived, protozoal, organism-derived, fungal, and bacterial immunogens, toxoids, toxins, polysaccharides, proteins, glycoproteins, peptides, cellular vaccines, such as using dendritic cells, DNA vaccines, recombinant proteins, glycoproteins, and peptides, and the like, for use in connection with, e.g., BCG, cholera, plague, typhoid, hepatitis A, B, and C, influenza A and B, parainfluenza, polio, rabies, measles, mumps, rubella, yellow fever, tetanus, diphtheria, hemophilus influenza b, tuberculosis, meningococcal and pneumococcal vaccines, adenovirus, HIV, chicken pox, cytomegalovirus, dengue, feline leukemia, fowl plague, HSV-1 and HSV-2, hog cholera, Japanese encephalitis, respiratory syncytial virus, rotavirus, papilloma virus, severe acute respiratory syndrome (SARS) virus

(coronavirus), anthrax, and yellow fever. Such additional agents can include, but are no limited to, drugs, such as antiviral agents or cytokines. The vaccine may be separate or may be physically or chemically linked to the IRM, such as by chemical conjugation or other means, so that they are delivered as a unit. TNFR agonists that may be delivered in conjunction with include, but are not limited to, CD40 receptor agonists.

The methods, materials, and articles of the present invention may be applicable for any suitable subject. Suitable subjects include, but are not limited to, animals such as, but not limited to, humans, non-human primates, rodents, dogs, cats, horses, pigs, sheep, goats, cows, or birds.

Suitable Immune Response Modifiers:

Immune response modifiers ("IRM") useful in the present invention include compounds that act on the immune system by inducing and/or suppressing cytokine biosynthesis. IRM compounds possess potent immunostimulating activity including, but not limited to, antiviral and antitumor activity, and can also down-regulate other aspects of the immune response, for example shifting the immune response away from a TH-2 immune response, which is useful for treating a wide range of TH-2 mediated diseases. IRM compounds can also be used to modulate humoral immunity by stimulating antibody production by B cells. Further, various IRM compounds have been shown to be useful as vaccine adjuvants (see, e.g., U.S. Pat. Nos. 6,083,505 and 6,406,705, and International Publication No. WO 02/24225).

In particular, certain IRM compounds effect their immunostimulatory activity by inducing the production and secretion of cytokines such as, e.g., Type I interferons, TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1, and/or MCP-1, and can also inhibit production and secretion of certain TH-2 cytokines, such as IL-4 and IL-5. Some IRM compounds are said to suppress IL-1 and TNF (U.S. Pat. No. 6,518,265).

For some embodiments, the preferred IRM compounds are so-called small molecule IRMs, which are relatively small organic compounds (e.g., molecular weight under about 1000 daltons, preferably under about 500 daltons, as opposed to large biologic protein, peptides, and the like).

Although not bound by any single theory of activity, some IRMs are known to be agonists of at least one Toll-like receptor (TLR). IRM compounds that are agonists for

TLRs selected from 6, 7, 8, and/or 9 may be particularly useful for certain applications. In some applications, for example, the preferred IRM compound is not a TLR7 agonist and is a TLR 8 or TLR 9 agonist. Some small molecule IRM compounds are agonists of TLRs such as 6, 7, and/or 8, while oligonucleotide IRM compounds are agonists of TLR9, and perhaps others. Thus, in some embodiments, the IRM that is attached to a particulate support material may be a compound identified as an agonist of one or more TLRs.

For example, without being bound to any particular theory or mechanism of action, IRM compounds that activate a strong cytotoxic lymphocyte (CTL) response may be particularly desirable as vaccine adjuvants, especially for therapeutic viral and/or cancer vaccines because a therapeutic effect in these settings is dependent on the activation of cellular immunity. For example, studies have shown that activation of T cell immunity in a given patient has a significant positive effect on the prognosis of the patient. Therefore the ability to enhance T cell immunity is believed to be critical to producing a therapeutic effect in these disease settings.

IRM compounds that are TLR 8 agonists may be particularly desirable for use with therapeutic cancer vaccines because antigen presenting cells that express TLR8 have been shown to produce IL-12 upon stimulation through TLR8. IL-12 is believed to play a significant role in activation of CTLs, which are important for mediating therapeutic efficacy as described above.

IRM compounds that are TLR 7 agonists and/or TLR 9 agonists may be particularly desirable for use with prophylactic vaccines because the type I interferon induced by stimulation through these TLRs is believed to contribute to the formation of neutralizing T_H1-like humoral and cellular responses.

IRM compounds that are both TLR 7 and TLR 8 agonists may be particularly desirable for use with therapeutic viral vaccines and/or cancer vaccines because TLR7 stimulation is believed to induce the production of type I IFN and activation of innate cells such as macrophages and NK cells, and TLR8 stimulation is believed to activate antigen presenting cells to initiate cellular adaptive immunity as described above. These cell types are able to mediate viral clearance and/or therapeutic growth inhibitory effects against neoplasms.

IRM compounds that are non-TLR 7 agonists, and do not induce substantial amounts of interferon alpha, may be desirable for use with certain vaccines such as

bacterial vaccines because TLR7 induces type I IFN production, which down-regulates the production of IL-12 from macrophages and DCs. IL-12 contributes to the subsequent activation of macrophages, NK cells and CTLs, all of which contribute to anti-bacterial immunity. Therefore the induction of anti-bacterial immunity against some kinds of bacteria may be enhanced in the absence of IFNa.

For purposes of the present application, one way to determine if an IRM compound is considered to be an agonist for a particular TLR is if it activates an NFkB/luciferase reporter construct through that TLR from the target species more than about 1.5 fold, and usually at least about 2 fold, in TLR transfected host cells such as, e.g., HEK293 or Namalwa cells relative to control transfectants. For information regarding TLR activation, see, e.g., International Publication Nos. WO 03/043573 and WO 03/043588, U.S. Patent Application Serial Nos. 10/777,310, 10/732,563, 10/732,796, and 10/788,731, U.S. Patent Publication No. US2004/0014779, and the other IRM patents and applications disclosed herein.

Preferred IRM compounds include a 2-aminopyridine fused to a five-membered nitrogen-containing heterocyclic ring.

Certain IRMs are small organic molecules (e.g., molecular weight under about 1000 Daltons, preferably under about 500 Daltons, as opposed to large biologic protein, peptides, and the like) such as those disclosed in, for example, U.S. Pat. Nos. 4,689,338; 4,929,624; 4,988,815; 5,037,986; 5,175,296; 5,238,944; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,367,076; 5,389,640; 5,395,937; 5,446,153; 5,482,936; 5,693,811; 5,741,908; 5,756,747; 5,939,090; 6,039,969; 6,083,505; 6,110,929; 6,194,425; 6,245,776; 6,331,539; 6,376,669; 6,451,810; 6,525,064; 6,545,016; 6,545,017; 6,558,951; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; European Patent 0 394 026; U.S. Patent Publication Nos. 2002/0016332; 2002/0055517; 2002/0110840; 2003/0133913; 2003/0199538; and 2004/0014779; and International Patent Publication Nos. WO 02/102377 and WO 03/103584.

Examples of classes of small molecule IRM compounds include, but are not limited to, derivatives of imidazoquinoline amines such as, for example, amide substituted imidazoquinoline amines, sulfonamide substituted imidazoquinoline amines, urea substituted imidazoquinoline amines, aryl ether substituted imidazoquinoline amines,

heterocyclic ether substituted imidazoquinoline amines, amido ether substituted imidazoquinoline amines, sulfonamido ether substituted imidazoquinoline amines, urea substituted imidazoquinoline ethers, thioether substituted imidazoquinoline amines, and 6-, 7-, 8-, or 9-aryl or heteroaryl substituted imidazoquinoline amines; tetrahydroimidazoquinoline amines including but not limited to amide substituted tetrahydroimidazoquinoline amines, sulfonamide substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline amines, aryl ether substituted tetrahydroimidazoquinoline amines, heterocyclic ether substituted tetrahydroimidazoquinoline amines, amido ether substituted tetrahydroimidazoquinoline amines, sulfonamido ether substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline ethers, and thioether substituted tetrahydroimidazoquinoline amines; imidazopyridine amines including but not limited to amide substituted imidazopyridine amines, sulfonamido substituted imidazopyridine amines, urea substituted imidazopyridine amines, aryl ether substituted imidazopyridine amines, heterocyclic ether substituted imidazopyridine amines, amido ether substituted imidazopyridine amines, sulfonamido ether substituted imidazopyridine amines, urea substituted imidazopyridine ethers, and thioether substituted imidazopyridine amines; 1,2-bridged imidazoquinoline amines; 6,7-fused cycloalkylimidazopyridine amines; imidazonaphthyridine amines; tetrahydroimidazonaphthyridine amines; oxazoloquinoline amines; thiazoloquinoline amines; oxazolopyridine amines; thiazolopyridine amines; oxazolonaphthyridine amines; thiazolonaphthyridine amines; and 1*H*-imidazo dimers fused to pyridine amines, quinoline amines, tetrahydroquinoline amines, naphthyridine amines, or tetrahydronaphthyridine amines.

Additional examples of small molecule IRMs include certain purine derivatives (such as those described in U.S. Pat. Nos. 6,376,501, and 6,028,076), certain imidazoquinoline amide derivatives (such as those described in U.S. Pat. No. 6,069,149), certain imidazopyridine derivatives (such as those described in U.S. Pat. No. 6,518,265), 1*H*-imidazopyridine derivatives (such as those described in Japanese Patent Application No. 9-255926), certain benzimidazole derivatives (such as those described in U.S. Pat. 6,387,938), certain derivatives of a 4-aminopyrimidine fused to a five membered nitrogen containing heterocyclic ring (such as adenine derivatives described in U.S. Pat. Nos. 6,376,501; 6,028,076 and 6,329,381; and in International Publication No. WO 02/08595),

and certain 3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine derivatives (such as those described in U.S. Patent Publication No. 2003/0199461). 1*H*-imidazopyridine derivatives (such as those described in U.S. Pat. No. 6,518,265 and European Patent Application EP No. 1 256 582)) are said to inhibit TNF and IL-1 cytokines.

Examples of small molecule IRMs that comprise a 4-aminopyrimidine fused to a five-membered nitrogen-containing heterocyclic ring include adenine derivatives (such as those described in U. S. Pat. Nos. 6,376,501; 6,028,076 and 6,329,381; and in International Publication No. WO 02/08595).

In some applications, for example, the preferred IRM compound is other than imiquimod or S-28463 (i.e., resiquimod: 4-Amino-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol).

Examples of particular IRM compounds include 2-propyl[1,3]thiazolo[4,5-*c*]quinolin-4-amine, which is considered predominantly a TLR 8 agonist (and not a substantial TLR 7 agonist), 4-amino-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol, which is considered predominantly a TLR 7 agonist (and not a substantial TLR 8 agonist), and 4-amino-2-(ethoxymethyl)-α,α-dimethyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol, which is a TLR 7 and TLR 8 agonist. In addition to its TLR 7 activity (and TLR 6 activity, but low TLR 8 activity), 4-amino- α,α -dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol has beneficial characteristics, including that it has a much lower CNS effect when delivered systemically compared to imiquimod. Other examples of specific IRM compounds include, e.g., N-[4-(4-amino-2-butyl-1*H*-imidazo[4,5-*c*][1,5]naphthyridin-1-yl)butyl]-N'-cyclohexylurea, 2-methyl-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*][1,5]naphthyridin-4-amine, 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*][1,5]naphthyridin-4-amine, N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethyl}methanesulfonamide, N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide, 2-methyl-1-[5-(methylsulfonyl)pentyl]-1*H*-imidazo[4,5-*c*]quinolin-4-amine, N-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide, 2-butyl-1-[3-(methylsulfonyl)propyl]-1*H*-imidazo[4,5-*c*]quinoline-4-amine, 2-butyl-1-{2-[(1-methylethyl)sulfonyl]ethyl}- 1*H*-imidazo[4,5-*c*]quinolin-4-amine, N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethyl}-N'-cyclohexylurea, N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-

dimethylethyl}cyclohexanecarboxamide, N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]ethyl}-N'-isopropylurea. Resiquimod, 4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol, may also be used in certain situations where a combination TLR 7 and TLR 8 agonist is desired.

Other IRM compounds include large biological molecules such as oligonucleotide sequences. Some IRM oligonucleotide sequences contain cytosine-guanine dinucleotides (CpG) and are described, for example, in U.S. Pat. Nos. 6,194,388; 6,207,646; 6,239,116; 6,339,068; and 6,406,705. Some CpG-containing oligonucleotides can include synthetic immunomodulatory structural motifs such as those described, for example, in U.S. Pat. Nos. 6,426,334 and 6,476,000. Other IRM nucleotide sequences lack CpG and are described, for example, in International Patent Publication No. WO 00/75304.

Various combinations of IRMs can be used if desired.

Exemplary Applications:

The metal-containing IRM-support complexes of the present invention can be used with a delivery device, particularly a high pressure (e.g., ballistic, or magnetic accelleration) delivery device, such as a gene gun, for delivery of the IRM. Typically, an IRM-support complex for use with delivery devices includes at least one IRM compound covalently attached to particulate support material including at least one metal (e.g., zero-valent transition metal), wherein the particulate support material has an average density of 10 g/cm^3 to 20 g/cm^3.

Delivery devices such as gene guns can be used for delivery of the IRM by propelling the IRM-support complex, which is contained in a reservoir in the delivery device, at cells or tissues at a speed sufficient for the particles to penetrate the surface barrier and become incorporated into the interior of the host. Such devices are disclosed, for example, in U.S. Pat. No. 5,371,015, for example. Other delivery devices are disclosed in U.S. Pat. No. 5,630,796 that can be used for delivery of powdered material using gas pressure to generate a supersonic gas flow. Such delivery devices are well-known to one of skill in the art.

The IRM-support complexes of the present invention are particularly useful for local delivery of an IRM. Local delivery of an IRM-support complex would allow for concentration of its biological activity to the site of application. Immobilization of the

IRM, as well as an antigen, would allow for maintaining these components at high concentration relative to one another without dilution into the periphery. In such applications, e.g., with an associated antigen, an IRM can be attached to a particle accompanied by a specific immunizing antigen on the same particle. Alternatively, an IRM can be attached to a particle while the immunizing antigen is attached to a second particle. The latter case would allow for admixture of the IRM-support complex with any one of many possible immunizing antigens. These could be administered simultaneously or sequentially with a delivery device, such as a gene gun. The initial targeted layers can be fine-tuned by the size and the density of the support materials and the applied force.

IRM-support complexes can also be used in deposition applications, particularly for inhalation into the lungs of a subject. For such applications, the particulate support material typically has an average particle size of 0.2 micron to 5 microns (preferably 2-5 microns), although larger particle sizes can be use as well. Targeted areas can include proximal, medial, or distal regions of the lungs. Selection of particle size would allow for zonal selectivity in deposition. For example, the 2-5 micron particles would allow for deposition into the distal airways of the lung. Larger particles would be limited to the proximal airways of the lung.

In certain embodiments, IRM-support complexes can also be used in targeting solid tumors. Typically, such particles have an average particle size of 5 nm (the permeable upper limit of a healthy blood vessel) to 100 nm (the permeable upper limit of a of tumor blood vessel). The particulate-IRM complex can be selectively delivered to the tumor site through the hypropermeated endothelium liner of the blood vessel.

The metal of the metal-containing IRM-support complex can be used for visualization of the location of deposition of the IRM-support material. Visualization, for example, can be accomplished by techniques such as x-ray or magnetic resonance imaging. The metal should be of sufficient electron density for the desired visualization technique. Typically, an IRM-support complex that can be visualized includes at least one metal-containing material (e.g., a zero-valent transition metal or metal oxide). Preferably, the metal is selected from the group consisting of Groups 6-11 and rare earth elements of the Periodic Table. Preferably, such complex has magnetic properties (preferably, superparamagnetic), fluorescent properties, or relatively high electron density. An ability

to visualize the administration of an IRM can be of advantage in monitoring the targeting of an IRM to a desired site.

If the support material is magnetic (e.g., either permanently magnetic, paramagnetic, or supermagnetic, preferably, superparamagnetic), an additional signal can result in magnetic resonance imaging. In certain embodiments, the resonance magnetic signal from the IRM-support complex can be recorded to generate 2- or 3-dimensional images and used as diagnostics for the host.

A magnetic metal-containing IRM-support complex (or just the metal after the IRM has detached, for example) can be further manipulated if desired. For example, the complex (or just the metal) can be relocated or redistributed inside the host by an external magnetic field to maximize the effects of the IRM. In some cases, the magnetic metal-containing IRM-support complex (or just the metal) can be removed from the host to minimize the long effect of the material by the external magnetic field, such as can be applied with a wearable magnetic collar.

The metal of the metal-containing IRM-support material can be used for absorption of energy from an external energy source (e.g., microwave) to break the linkage with the IRM and release the IRM. For example, an IRM can be covalently bonded to a single-stranded oligonucleotide, which can hybridize with the complementary oligonucleotide immobilized on the support. Upon the absorption of microwave energy from an external energy source, the temperature would increase to denature the oligonucleotide (see, for example, J. Nam et al., Science, 301, 1884-1886 (2003)) and release the IRMs as desired.

The metal-containing IRM-support complex can be used in a wide variety of applications, such as in the treatment of a wide variety of conditions. For example, IRMs such as imiquimod - a small molecule, imidazoquinoline IRM, marketed as ALDARA (3M Pharmaceuticals, St. Paul, MN) - have been shown to be useful for the therapeutic treatment of warts, as well as certain cancerous or pre-cancerous lesions (See, e.g., Geisse *et al.*, *J. Am. Acad. Dermatol.*, 47(3): 390-398 (2002); Shumack *et al.*, *Arch. Dermatol.*, 138: 1163-1171 (2002); U.S. Pat. No. 5,238,944 and International Publication No. WO 03/045391.

Conditions that may be treated by administering an IRM-support complex of the present invention include, but are not limited to:

(a) viral diseases such as, for example, diseases resulting from infection by an adenovirus, a herpesvirus (e.g., HSV-I, HSV-II, CMV, or VZV), a poxvirus (e.g., an orthopoxvirus such as variola or vaccinia, or molluscum contagiosum), a picornavirus (e.g., rhinovirus or enterovirus), an orthomyxovirus (e.g., influenzavirus), a paramyxovirus (e.g., parainfluenzavirus, mumps virus, measles virus, and respiratory syncytial virus (RSV)), a coronavirus (e.g., SARS), a papovavirus (e.g., papillomaviruses, such as those that cause genital warts, common warts, or plantar warts), a hepadnavirus (e.g., hepatitis B virus), a flavivirus (e.g., hepatitis C virus or Dengue virus), or a retrovirus (e.g., a lentivirus such as HIV);

(b) bacterial diseases such as, for example, diseases resulting from infection by bacteria of, for example, the genus Escherichia, Enterobacter, Salmonella, Staphylococcus, Shigella, Listeria, Aerobacter, Helicobacter, Klebsiella, Proteus, Pseudomonas, Streptococcus, Chlamydia, Mycoplasma, Pneumococcus, Neisseria, Clostridium, Bacillus, Corynebacterium, Mycobacterium, Campylobacter, Vibrio, Serratia, Providencia, Chromobacterium, Brucella, Yersinia, Haemophilus, or Bordetella;

(c) other infectious diseases, such chlamydia, fungal diseases including but not limited to candidiasis, aspergillosis, histoplasmosis, cryptococcal meningitis, or parasitic diseases including but not limited to malaria, pneumocystis carnii pneumonia, leishmaniasis, cryptosporidiosis, toxoplasmosis, and trypanosome infection; and

(d) neoplastic diseases, such as intraepithelial neoplasias, cervical dysplasia, actinic keratosis, basal cell carcinoma, squamous cell carcinoma, renal cell carcinoma, Kaposi's sarcoma, melanoma, renal cell carcinoma, leukemias including but not limited to myelogeous leukemia, chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, B-cell lymphoma, and hairy cell leukemia, and other cancers;

(e) T_H2-mediated, atopic diseases, such as atopic dermatitis or eczema, eosinophilia, asthma, allergy, allergic rhinitis, and Ommen's syndrome;

(f) certain autoimmune diseases such as systemic lupus erythematosus, essential thrombocythaemia, multiple sclerosis, discoid lupus, alopecia areata; and

(g) diseases associated with wound repair such as, for example, inhibition of keloid formation and other types of scarring (e.g., enhancing would healing, including chronic wounds).

Additionally, an IRM-support complex of the present invention may be useful as a vaccine adjuvant for use in conjunction with any material that raises either humoral and/or cell mediated immune response, such as, for example, live viral, bacterial, or parasitic immunogens; inactivated viral, tumor-derived, protozoal, organism-derived, fungal, or bacterial immunogens, toxoids, toxins; self-antigens; polysaccharides; proteins; glycoproteins; peptides; cellular vaccines; DNA vaccines; autologous vaccines; recombinant proteins; glycoproteins; peptides; and the like, for use in connection with, for example, BCG, cholera, plague, typhoid, hepatitis A, hepatitis B, hepatitis C, influenza A, influenza B, parainfluenza, polio, rabies, measles, mumps, rubella, yellow fever, tetanus, diphtheria, hemophilus influenza b, tuberculosis, meningococcal and pneumococcal vaccines, adenovirus, HIV, chicken pox, cytomegalovirus, dengue, feline leukemia, fowl plague, HSV-1 and HSV-2, hog cholera, Japanese encephalitis, respiratory syncytial virus, rotavirus, papilloma virus, yellow fever, and Alzheimer's Disease.

Certain IRM-support complexes of the present invention may be particularly helpful in individuals having compromised immune function. For example, certain complexes may be used for treating the opportunistic infections and tumors that occur after suppression of cell mediated immunity in, for example, transplant patients, cancer patients and HIV patients.

Particulate Support Material:

Selection of a particulate support material to serve as a substrate for attachment of an IRM can vary widely within the scope of the invention. A particulate support material can be porous or nonporous, depending on preferred final use. A particulate support material can be made of a variety of materials as long as a portion of it includes a metal. The metal can be coated on or impregnated in particles of another material. The metal can form the core of the particulate support material. For example, such particulate support material includes substrates made of inorganic or organic materials, typically polymeric materials, or combinations of materials, as long as they include (e.g., are coated with or impregnated with) a metal (e.g., a transition metal, metalloid, or a rare earth metal), which can be a zero-valent metal (although this is not a requirement). The inorganic particles can be made of metal oxides (e.g., TiO_2 or SiO_2) and can be in the form of ceramics (e.g., alumina or zirconia) or glasses, for example. Other compounds or complexes containing a metal, whether it be in a zero-valent oxidation state or not, can be used as particles in the present invention.

In certain embodiments, the selected particulate support materials, such as iron oxide or ferritin, can eventually be degraded, broken down, or secreted by the host after a desired duration.

In certain embodiments, the selected particulate support materials, such superparamagnetic beads, can be energized by an external magnetic source, which makes remote manipulation possible.

In certain embodiments, the selected particulate support materials, such metal oxide, can be heated by a remote energy source such as microwaves.

Ceramic, glass, and metallic particulate materials are all known in the art and are commercially available or can be prepared by a variety of known techniques. For example, a variety of colloidal gold particles are available commercially from ICN Biomedicals, Inc., Aurora, OH. Magnetic beads (such as those available under the trade designation DYNABEADS), metal particles, and metal oxides, are available from Dynal Biotech (Lake Success, NY), Argonide (Sanford, NY), and NanoSource Technologies (Oklahoma City, OK). A variety of silica particles are available from Naclo, Naperville, IL. Silica coated superparamagnetic particles are available from Chemicell Gmbh, Berlin,

Germany). Also suitable are quantum dots, such as CdSe particles, which typically have a particle size of 10 nm or less, and often have a particle size of 2 nm to 5 nm.

Suitable polymers for use in the particulate support material may be natural or synthetic polymers. The polymers can form the core of the particles with a metal coated thereon or the polymers can form a coating on a metal core material. Methods for making metal-coated particles include, for example, metal plasma vacuum deposition, and electric plating. Methods for making polymer-coated metal particles include, for example, solvent coating, and techniques for preparing self-assembled monolayers. Such methods are well-known to one of skill in the art.

Synthetic polymers are preferred. Herein, a polymer includes homopolymers and copolymers. A copolymer is used to refer to a polymer prepared from two or more monomers, and includes terpolymers, tetrapolymers, etc.

Exemplary synthetic polymers include, but are not limited to: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols (i.e., polyalkylene oxides), polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butyl methacrylate), poly(isobutyl methacrylate), poly(hexyl methacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), poly(vinyl acetate), poly(vinyl chloride), polystyrene, polyamides, polyvinylpyrrolidone, and polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-cocaprolactone), and fluorinated polymers.

Exemplary natural polymers include, but are not limited to: alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof

(substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), zein, and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. Copolymers and mixtures of any of these polymers could be used if desired. Polysciences, Inc. (Warrington, PA) supplies many types of polymeric beads.

Other examples of particulate support materials include, but are not limited to, carbohydrate beads and latex beads, such as those commercially available from many suppliers, including, for example Biorad and Pierce. The particles can also be in the form of microparticles, such as microspheres, microcapsules, etc. The particles can be quantum dots.

The particles having a metal and an IRM associated therewith can include a combination of materials. For example, they can include a combination of inorganic and organic materials. This can occur by layering the materials, for example. One or more of the materials can be associated (e.g., attached) to the particulate support material on the outermost surface such that an IRM is masked or hidden from a body's immune system until it reaches its targeted site of action. For example, gold particles having one or more IRMs attached thereto can have a coating of a polyalkylene oxide polymer (e.g., polyethylene glycol) thereon (see, e.g., Gref et al., Colloids and Surfaces B: Biointerfaces 18, 301-313, 2000). The polyalkylene oxide can function to mask the IRM from the body's immune system until it reaches its targeted site of action.

Attachment to Substrates:

IRMs can be attached to a particulate support material through either covalent attachment or non-covalent attachment. Non-covalent attachment of an IRM to a particulate support material includes attachment by ionic interaction or hydrogen bonding, for example.

One example of a non-covalent attachment included in the present invention is the well-know biotin-avidin system. Avidin-biotin affinity-based technology has found wide applicability in numerous fields of biology and biotechnology since the pioneering work by Dr. Edward Bayer and Dr. Meier Wilchek in the 1970's. The affinity constant between avidin and biotin is remarkably high (the dissociation constant, Kd, is approximately 10^{-15} M, see, Green, Biochem. J., 89, 599, 1963) and is not significantly lessened when biotin is

coupled to a wide variety of biomolecules. Numerous chemistries have been identified for coupling biomolecules to biotin with minimal or negligible loss in the activity or other desired characteristics of the biomolecule. A review of the biotin-avidin technology can be found in Applications of Avidin-Biotin Technology to Affinity-Based Separation, Bayer, et al., J. of Chromatography, 1990, pgs. 3-11.

Streptavidin, and its functional homolog avidin, are tetrameric proteins, having four identical subunits. Streptavidin is secreted by the actinobacterium Streptomyces avidinii. A monomer of streptavidin or avidin contains one high-affinity binding site for the water-soluble vitamin biotin and a streptavidin or avidin tetramer binds four biotin molecules.

Biotin, also known as vitamin H or cis-hexahydro-2-oxo-1*H*-thieno-[3,4]-imidazole-4-pentanoic acid, is a basic vitamin which is essential for most organisms including bacteria and yeast. Biotin has a molecular weight of about 244 daltons, much lower than its binding partners avidin and streptavidin. Biotin is also an enzyme cofactor of pyruvate carboxylase, trans-carboxylase, acetyl-CoA-carboxylase and beta-methylcrotonyl-CoA carboxylase which together carboxylate a wide variety of substrates.

Both streptavidin and avidin exhibit extremely tight and highly specific binding to biotin which is one of the strongest known non-covalent interactions between proteins and ligands, with a molar dissociation constant of 10^{-15} molar (M) (Green, Advances in Protein Chemistry, Vol. 29, pp. 85-133, 1975), and a t1/2 of ligand dissociation of 89 days (Green, N.M., Advances in Protein Chemistry, Vol. 29, pp. 85-133, 1975). The avidin-biotin bond is stable in serum and in the circulation (Wei et al., Experientia, Vol. 27, pp. 366-368, 1970). Once formed, the avidin-biotin complex is unaffected by most extremes of pH, organic solvents and denaturing conditions. Separation of streptavidin from biotin requires conditions, such as 8M guanidine, pH 1.5, or autoclaving at 121°C for 10 minutes (min).

IRMs may be biotinylated using any known methodologies. For example, IRMs may be biotinylated chemically, using activated biotin analogues, such as N-hydroxysuccinimidobiotin (NHS-biotin), which is commercially available from Pierce Chemical Company, Rockford, IL, and requires the presence of a free primary amino group on the IRM.

Representative methods for covalent attaching an IRM to a particulate support material include chemical crosslinkers, such as heterobifunctional crosslinking compounds

(i.e., "linkers") that react to form a bond between reactive groups (such as hydroxyl, amino, amido, or sulfhydryl groups) in a the immune response modifier and other reactive groups (of a similar nature) in the support material. This bond may be, for example, a peptide bond, disulfide bond, thioester bond, amide bond, thioether bond, and the like.

Immune response modifiers may be covalently bonded to a particulate support material by any of the methods known in the art. For example, U.S. Pat. Nos. 4,722,906, 4,979,959, 4,973,493, and 5,263,992 relate to devices having biocompatible agents covalently bound via a photoreactive group and a chemical linking moiety to the biomaterial surface. U.S. Pat. Nos. 5,258,041 and 5,217,492 relate to the attachment of biomolecules to a surface through the use of long chain chemical spacers. U.S. Pat. Nos. 5,002,582 and 5,263,992 relate to the preparation and use of polymeric surfaces, wherein polymeric agents providing desirable properties are covalently bound via a photoreactive moiety to the surface. Others have used photochemistry to modify the surfaces of biomedical devices, e.g., to coat vascular grafts. (See, e.g., Kito et al., ASAIO Journal 39, M506-M511, 1993; and Clapper et al., Trans. Soc. Biomat. 16, 42, 1993). Cholakis and Sefton synthesized a polymer having a polyvinyl alcohol (PVA) backbone and heparin bioactive groups. The polymer was coupled to polyethylene tubing via nonlatent reactive chemistry, and the resultant surface was evaluated for thromboresistance in a series of in vitro and in vivo assays (Cholakis et al., J. Biomed. Mater. Res., 23, 399-415, 1989 and Cholakis et al., J. Biomed. Mater. Res., 23, 417-441, 1989). Finally, Kinoshita et al. disclose the use of reactive chemistry to generate polyacrylic acid backbones on porous polyethylene, with collagen molecules being subsequently coupled to carboxyl moieties on the polyacrylic acid backbones. (See Kinoshita et al., Biomaterials 14, 209-215, 1993). U.S. Pat. No. 6,127,448 discusses the preparation of biocompatible polymeric coatings.

In a preferred embodiment, the IRM can be attached to a particulate support material using a linking group. The linking group can be any suitable organic linking group that allows the substrate to be covalently coupled to the immune response modifier moiety while preserving an effective amount of IRM activity. In some embodiments, the linking group may be selected to create sufficient space between the active core of the immune response modifier moiety and the substrate that the substrate does not interfere with a biologically effective interaction between the active core and the T cells that results in IRM activity such as cytokine production.

The linking group includes a reactive group capable of reacting with a reactive group on the substrate to form a covalent bond. Suitable reactive groups include those discussed in Hermanson, Bioconjugate Techniques, Academic Press, Chapter 2 "The Chemistry of Reactive Functional Groups", 137-166, 1996. For example, the linking group may react with a primary amine (e.g., an N-hydroxysuccinimidyl ester or an N-hydroxysulfosuccinimidyl ester); it may react with a sulfhydryl group (e.g., a maleimide or an iodoacetyl), or it may be a photoreactive group (e.g. a phenyl azide including 4-azidophenyl, 2-hydroxy-4-azidophenyl, 2-nitro-4-azidophenyl, and 2-nitro-3-azidophenyl). The linking group may also be an alkoxysilyl group (e.g., a triethyoxysilyl group) that can be covalently coupled to an IRM. The alkoxysilyl group can then be covalently coupled to a silicon-containing particulate support material such as silica particles.

The substrate includes a chemically active group accessible for covalent coupling to the linking group. A chemically active group accessible for covalent coupling to the linking group includes groups that may be used directly for covalent coupling to the linking group or groups that may be modified to be available for covalent coupling to the linking group. For example, suitable chemically active groups include, but are not limited to, primary amines and sulfhydryl groups.

Typically, attachment may occur by reacting an immune response modifier with a crosslinker and then reacting the resulting intermediate with a substrate. Many crosslinkers suitable for preparing bioconjugates are known and many are commercially available. See for example, Hermanson, Bioconjugate Techniques, Academic Press, 1996.

Attachment also may occur, for example, according to the method shown in Reaction Scheme I in which the substrate is linked to the IRM moiety through R_1. In step (1) of Reaction Scheme I a compound of Formula III is reacted with a heterobifunctional crosslinker of Formula IV to provide a compound of II. R_A and R_B each contain a functional group that is selected to react with the other. For example, if R_A contains a primary amine, then a heterobifunctional crosslinker may be selected in which R_B contains an amine-reactive functional group such as an N-hydroxysulfosuccinimidyl ester. R_A and R_B may be selected so that they react to provide the desired linker group in the conjugate.

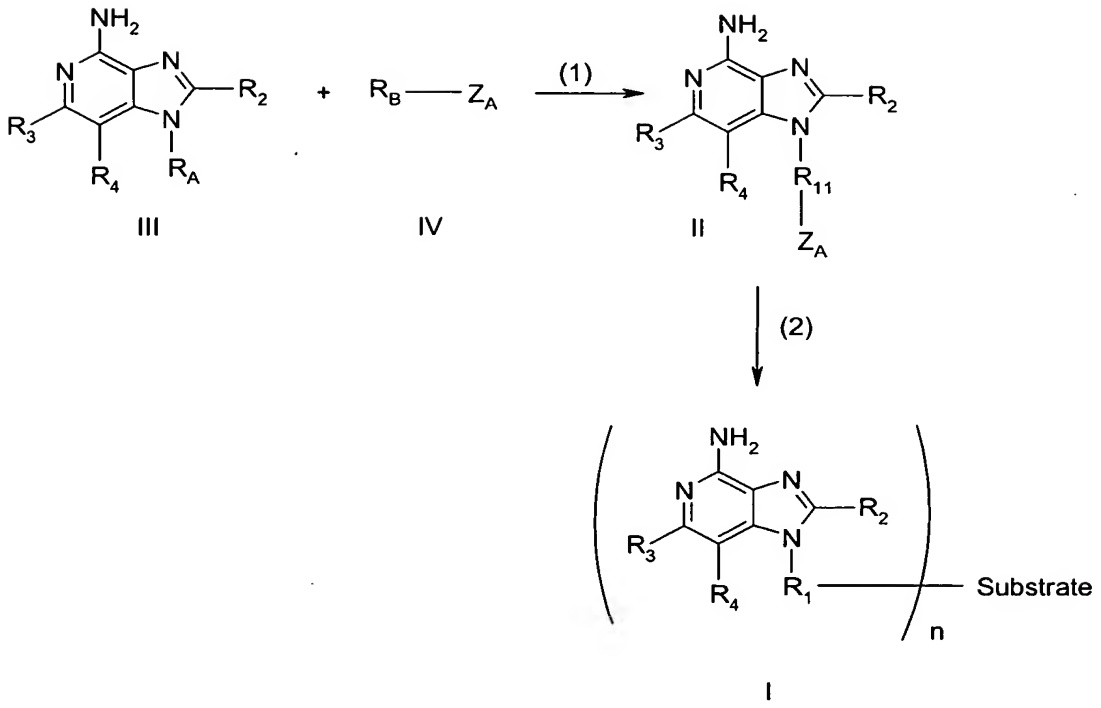
Methods for preparing compounds of Formula III where R_A contains a functional group are known. See, for example, U.S. Pat. Nos. 4,689,338; 4,929,624; 5,268,376;

5,389,640; 5,352,784; 5,494,916; 4,988,815; 5,367,076; 5,175,296; 5,395,937; 5,741,908; 5,693,811; 6,069,149; 6,194,425; 6,331,539; 6,451,810; 6,525,064; 6,541,485; 6,545,016; 6,545,017; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; and International Publication No. WO 03/103584.

Many heterobifunctional crosslinkers are known and many are commercially available. See for example, Hermanson, Bioconjugate Techniques, Academic Press, Chapter 5 "Heterobifunctional Cross-Linkers", 229-285, 1996. The reaction generally can be carried out by combining a solution of the compound of Formula III in a suitable solvent such as N,N-dimethylformamide with a solution of the heterobifunctional cross-linker of Formula IV in a suitable solvent such as N,N-dimethylformamide. The reaction may be run at ambient temperature. The product of Formula II may then be isolated using conventional techniques.

In step (2) of Reaction Scheme I a compound of Formula II that contains reactive group Z_A is reacted with the substrate to provide the IRM-couples substrate of Formula I. In one embodiment the reaction can be carried out by combining a solution of the compound of Formula II in a suitable solvent such as dimethyl sulfoxide with the substrate. The reaction may be run at ambient temperature or at a reduced temperature (approximately 4°C). If Z_A is a photoreactive group such as a phenyl azide then the reaction mixture will be exposed to long wave UV light for a length of time adequate to effect cross-linking (e.g., 10 – 20 minutes). The average number of immune response modifier moieties per substrate surface area may be controlled by adjusting the amount of compound of Formula II used in the reaction.

Reaction Scheme I



Alternatively, a compound of Formula II may be synthesized without using a heterobifunctional crosslinker. So long as the compound of Formula II contains the reactive group Z_A, it may be reacted with the substrate using the method of step (2) above to provide an IRM-coupled substrate.

The R groups can be hydrogen or organic groups that can optionally include various substitutions. They can include alkyl groups, alkenyl groups, including haloalkyl groups, aryl groups, heteroaryl groups, heterocyclyl groups, and the like.

For example, preferred R_2 groups include hydrogen, alkyl groups having 1 to 4 carbon atoms (i.e., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, and cyclopropylmethyl), and alkoxyalkyl groups (e.g., methoxyethyl and ethoxymethyl). Preferably R_3 and R_4 are independently hydrogen or methyl or R_3 and R_4 join together to form a benzene ring, a pyridine ring, a 6-membered saturated ring or a 6-membered saturated ring containing a nitrogen atom. One or more of these preferred substituents, if present, can be present in the compounds of the invention in any combination.

As used herein, the terms "alkyl," "alkenyl," and the prefix "alk-" include straight chain, branched chain, and cyclic groups, i.e. cycloalkyl and cycloalkenyl. Unless otherwise specified, these groups contain from 1 to 20 carbon atoms, with alkenyl groups containing from 2 to 20 carbon atoms. Preferred groups have a total of up to 10 carbon atoms. Cyclic groups can be monocyclic or polycyclic and preferably have from 3 to 10 ring carbon atoms. Exemplary cyclic groups include cyclopropyl, cyclopentyl, cyclohexyl, cyclopropylmethyl, and adamantyl.

The term "haloalkyl" is inclusive of groups that are substituted by one or more halogen atoms, including perfluorinated groups. This is also true of groups that include the prefix "halo-". Examples of suitable haloalkyl groups are chloromethyl, trifluoromethyl, and the like.

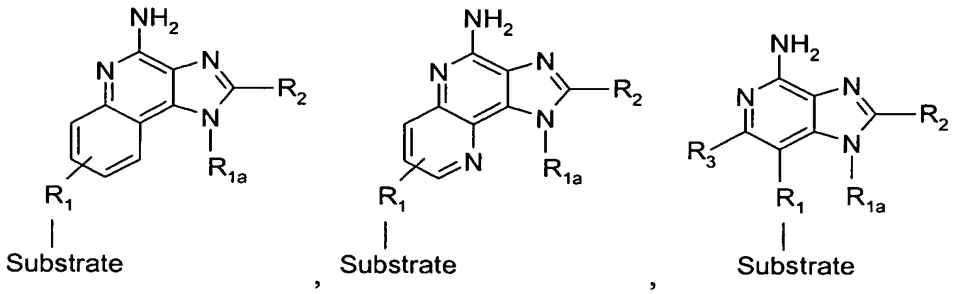
The term "aryl" as used herein includes carbocyclic aromatic rings or ring systems. Examples of aryl groups include phenyl, naphthyl, biphenyl, fluorenyl and indenyl. The term "heteroaryl" includes aromatic rings or ring systems that contain at least one ring hetero atom (e.g., O, S, N). Suitable heteroaryl groups include furyl, thienyl, pyridyl, quinolinyl, isoquinolinyl, indolyl, isoindolyl, triazolyl, pyrrolyl, tetrazolyl, imidazolyl, pyrazolyl, oxazolyl, thiazolyl, benzofuranyl, benzothiophenyl, carbazolyl, benzoxazolyl, pyrimidinyl, benzimidazolyl, quinoxalinyl, benzothiazolyl, naphthyridinyl, isoxazolyl, isothiazolyl, purinyl, quinazolinyl, and so on.

"Heterocyclyl" includes non-aromatic rings or ring systems that contain at least one ring hetero atom (e.g., O, S, N) and includes all of the fully saturated and partially unsaturated derivatives of the above mentioned heteroaryl groups. Exemplary heterocyclic groups include pyrrolidinyl, tetrahydrofuranyl, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, thiazolidinyl, isothiazolidinyl, and imidazolidinyl.

The aryl, heteroaryl, and heterocyclyl groups can be unsubstituted or substituted by one or more substituents independently selected from the group consisting of alkyl, alkoxy, methylenedioxy, ethylenedioxy, alkylthio, haloalkyl, haloalkoxy, haloalkylthio, halogen, nitro, hydroxy, mercapto, cyano, carboxy, formyl, aryl, aryloxy, arylthio, arylalkoxy, arylalkylthio, heteroaryl, heteroaryloxy, heteroarylthio, heteroarylalkoxy, heteroarylalkylthio, amino, alkylamino, dialkylamino, heterocyclyl, heterocycloalkyl, alkylcarbonyl, alkenylcarbonyl, alkoxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylcarbonyl, heteroarylcarbonyl, aryloxycarbonyl,

heteroaryloxycarbonyl, arylthiocarbonyl, heteroarylthiocarbonyl, alkanoyloxy, alkanoylthio, alkanoylamino, arylcarbonyloxy, arylcarbonythio, alkylaminosulfonyl, alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aryldiazinyl, alkylsulfonylamino, arylsulfonylamino, arylalkylsulfonylamino, alkylcarbonylamino, alkenylcarbonylamino, arylcarbonylamino, arylalkylcarbonylamino, heteroarylcarbonylamino, heteroarylalkycarbonylamino, alkylsulfonylamino, alkenylsulfonylamino, arylsulfonylamino, arylalkylsulfonylamino, heteroarylsulfonylamino, heteroarylalkylsulfonylamino, alkylaminocarbonylamino, alkenylaminocarbonylamino, arylaminocarbonylamino, arylalkylaminocarbonylamino, heteroarylaminocarbonylamino, heteroarylalkylaminocarbonylamino and, in the case of heterocyclyl, oxo. If other groups are described as being "substituted" or "optionally substituted," then those groups can also be substituted by one or more of the above-enumerated substituents.

In Reaction Scheme I the IRM is attached to the substrate through a linking group at the N^1 nitrogen of the imidazole ring. Alternatively the linking can occur at different positions on the ring system. Examples of which are shown below for imidazoquinoline amines, imidazonaphthyridine amines and imidazopyridine amines respectively.



The attachment is effected using the method of Reaction Scheme I starting with an IRM containing reactive group R_A at the desired attachment point.

An amount of an IRM-support complex effective for a given therapeutic or prophylactic application is an amount sufficient to achieve the intended therapeutic or prophylactic application. The precise amount of IRM-support complex used will vary according to factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the particulate support material, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, and the

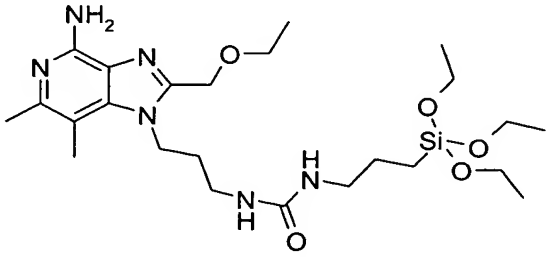
species to which the formulation is being administered. Accordingly it is not practical to set forth generally the amount that constitutes an amount of IRM-support complex effective for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

The dosing regimen may depend at least in part on many factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the particulate support material, the amount of IRM being administered, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM-support complex, and the species to which the formulation is being administered. Accordingly it is not practical to set forth generally the dosing regimen effective for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

EXAMPLES

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular materials and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention.

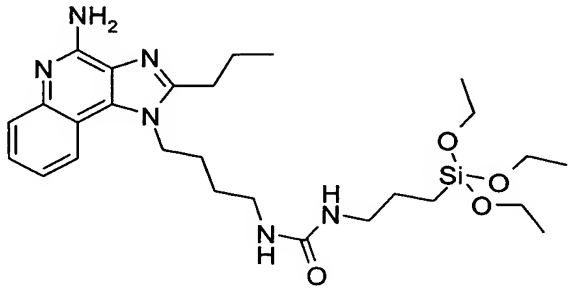
Preparation of *N*-[3-(4-Amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea



Into a flask was placed 1-(3-aminopropyl)-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine (100 milligrams (mg), 0.36 millimole (mmol); Example 21 in U.S. Pat. No. 6,545,016) and 5 milliliters (mL) anhydrous dimethyl sulfoxide (DMSO).

The mixture was stirred until the solid was completely dissolved. To the solution was slowly added 3-(triethoxysilyl) propyl isocyanate (89.1 mg, 0.36 mmol) in DMSO (1.5 mL) at room temperature. After the addition, the reaction solution was stirred overnight. The reaction solution was sampled and analyzed by NMR. The spectra showed the desired addition product, *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea, at 100% conversion. The sample was also analyzed by liquid chromatography, the spectrum showed a single product peak with the disappearance of the starting materials.

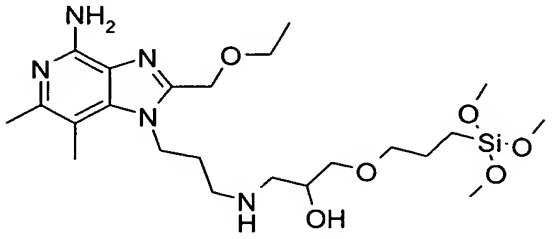
Preparation of *N*-[4-(4-Amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea



Into a flask was placed 1-(4-aminobutyl)-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (100 mg, 0.336 mmol; which can be prepared using the methods disclosed in U.S. Pat. No. 6,069,149) and 5 mL anhydrous dimethyl sulfoxide (DMSO). The mixture was stirred until the solid was completely dissolved. To the solution was slowly added 3-(triethoxysilyl) propyl isocyanate (83.2 mg, 0.336 mmol) in DMSO (1.5 mL) at room temperature. After the addition, the reaction solution was stirred overnight. The reaction solution was sampled and analyzed by NMR. The spectra showed the desired addition product, *N*-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea, at 100% conversion. The sample was also analyzed by liquid chromatography, the spectrum showed a single product peak with the disappearance of the starting materials.

The reaction was repeated using 15 mL of anhydrous tetrahydrofuran (THF) in place of the DMSO. Analysis of the resulting product by NMR showed 97% conversion of the starting material to the desired addition product.

Preparation of 2-ethoxymethyl-1-((3-{2-hydroxy-3-[3-(trimethoxysilyl)propoxy]propyl}amino))propyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridine-4-amine



Into a flask was placed 1-(3-aminopropyl)-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine (10 mg, 0.036 mmol; Example 21 in U.S. Pat. No. 6,545,016) and 2.5 mL anhydrous tetrahydrofuran. The mixture was stirred until the solid was completely dissolved. To the solution was slowly added 3-glycidoxypropyltrimethoxysilane (8.51 mg, 0.036 mmol) at room temperature. After the addition, the reaction solution was stirred overnight. The reaction solution was sampled and analyzed by NMR. The spectra showed the desired addition product, 2-ethoxymethyl-1-((3-{2-hydroxy-3-[3-(trimethoxysilyl)propoxy]propyl}amino))propyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridine-4-amine, at 100% conversion.

Example 1:

IRMs were covalently coupled to gold particles to form nanometer-sized IRM-gold conjugates through a two-step reaction: the gold surface was functionalized with carbonate by reacting with thiol carbonate; the carbonate functional group was then coupled to the primary amine group of an IRM catalyzed by a carbodiimide.

Briefly, ten micro-liters of 100 mM solution of mercaptoacetic acid (catalog no. 10,900-2, Aldrich, Milwaukee, WI) were added to one mL of colloidal gold particles solution (approximately 10 nanomolar (nM), catalog no. 154015, average size = 40 nm, from ICN Biomedicals Inc., Aurora, OH). Under a nitrogen atmosphere, the mixture was shaken at 3 Hz for 3 hours (hr) at room temperature.

Twenty micro-liters of 10 mg/L PBS buffer (pH 7.2) solution of an imidazoquinoline IRM compound (4-amino-2-ethoxymethyl-1*H*-imidazo[4,5-c]quinoline-

1-ethanamine, disclosed in U.S. Pat. No. 6,069,149), 20 microliters of 50 milligrams per liter (mg/L) PBS buffer solution of 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) (EDC, HCl salt, Pierce, Rockford, IL), and one drop of approximately 1N HCl, were then added to the mixture. The final mixture was shaken at 3 Hz at room temperature for another 12 hours (hr) followed by centrifugation at 14,000 revolutions per minute (rpm) for 30 minutes (min). After removing the supernatant, the precipitant was washed with 0.5 mL of PBS buffer twice before being redispersed in 1 millilter (mL) of PBS. A field emission SEM micrograph showed that, the modified particles were well separated and distributed. The infrared spectrum showed that there was a significant increase at the -NH- signal, indicating the successful coupling of IRM to the colloidal gold.

Example 2:

Similarly, a gold conjugate was also made with 10 nm colloidal gold (catalog number154011, ICN Biomedicals).

Example 3:

IRM-gold particles were also made from avidin-biotin or anti biotin-biotin coupling: reacting the commercially available gold-strepavidin (Amersham Biosciences, Nanoprobes, Inc. Stoney Brook, NY) or anti-biotin Nanogold Fab' conjugate (Nanoprobes, Inc. Stoney Brook, NY) with the biotin complex of Example 29 of U.S. Pat. No. 6,451,810, which is comparable to the uncomplexed IRM in stimulating TNF release, but superior in IL-6 stimulation.

Example 4:

An IRM conjugate of ferritin, a metaloprotein containing 4000 to 5000 Fe^{3+} ions, was synthesized through direct coupling between the carboxyl group of [(4-amino-1-isobutyl-1*H*-imidazo[4,5-c]quinolin-2-yl)methoxy]acetic acid and the primary amine of ferritin catalyzed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).

Five milliliters of a solution of [(4-amino-1-isobutyl-1*H*-imidazo[4,5-c]quinolin-2-yl)methoxy]acetic acid in pH 7.4 PBS buffer (0.4 g/L) was added to a mixture of 3 ml of 50 g/L of ferritin in pH 7.4 PBS buffer solution from ICN Biochemedicals Inc., Aurora, OH, 2 mL of freshly made 20 mM EDC in PBS, and 10 drops of 1N HCl. After a 5-

second vortex mixing, the mixture was allowed to react overnight. The mixture was then eluted through a size-exclusion liquid chromatography (PD-10) column. The brown-colored fraction was collected. The average ratio of [(4-amino-1-isobutyl-1*H*-imidazo[4,5-c]quinolin-2-yl)methoxy]acetic acid to ferritin in the conjugate was determined to be 0.6 (M/M), based on the UV spectrum measurement of [(4-amino-1-isobutyl-1*H*-imidaz[4,5-c]quinolin-2-yl)methoxy]acetic acid in the initial solution and the eluted solution. The recovery rate of ferritin was 95% after passing through the column. The eluted fraction was verified by HPLC, which showed a single peak. No significant lost of iron was observed during the modification. The conjugate showed biological activities in a test with RAW cells.

Example 5:

An IRM was covalently immobilized onto functionalized superparamagnetic particles using a modified protocol based on the manufacturer's suggested protocol. Briefly, one hundred milligrams of freeze-dried DYNABEADS M-270 Epoxy (from Dynal Biotect, Lake Success, NY, containing approximately 6.7×10^9 beads) was suspended in 7 mL of de-ionized water. After being vortexed for 30 seconds and incubated for 10 minutes, the mixture was centrifuged at 3000 Gravity (G) for 10 min and the supernatant was discarded.

Three milliliters of a freshly prepared solution (0.4 grams per liter (g/L)) of 1-(4-aminobutyl)-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (which can be prepared using the methods disclosed in U.S. Pat. No. 6,069,149) in carbonate-bicarbonate buffer (0.1 M, pH 9.4) and 5 mL of 4 M ammonium sulfate in de-ionized water were added to the beads. The mixture was vortexed for 30 seconds and then placed on a shaker operating at 10 Hz at room temperature for 24 hours.

The mixture was centrifuged at 3000 gauss (G) for 10 min. The supernatant was removed and the IRM concentration was determined by UV absorption at 247 nm. The beads were washed with 7 mL of methanol 3 times and 7 ml of Dulbecco's PBS 3 times. The IRM content in the modified beads was calculated by subtracting the amount of IRM found in the supernatant and washes from the amount of IRM that was initially combined with the beads.

Example 6: An IRM was covalently immobilized onto nanosized superparamagnetic particles using the following procedure.

A portion (0.1 mL) of water-based ferrofluid (EMG 304, Nashua, NH), a water based dispersion of iron oxide particles with dimensions in the range of 5-15 nm, was diluted with 4 mL de-ionized water and 20 mL 2-propanol. Under continuous mechanical stirring, 0.3 mL ammonia (30 wt-%, Aldrich) and 8.5 mg of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea was slowly added to the dispersion. The reaction was allowed to proceed at room temperature for 4 hours under continuous stirring. After the reaction was complete, the IRM-attached magnetic particles were concentrated using a magnet.

Example 7:

An IRM was covalently attached to core shell superparamagnetic particles using the following procedure. A portion (1 mL) of water-based silica coated superparamagnetic particles (50 mg, SiMAG-1, Chemicell Gmbh, Berlin, Germany) a water based dispersion of core shell magnetic particles with dimensions in the range of 100 nm, was diluted with 5 mL de-ionized water and 15 mL 2-propanol. Under continuous mechanical stirring, 0.3 mL ammonia (30 wt-%, Aldrich) and 8.5 mg of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea was slowly added to the dispersion. The reaction was allowed to proceed at room temperature for 4 hours under continuous stirring. After the reaction was complete, the IRM-attached magnetic particles were concentrated using a magnet.

Example 8: Preparation of IRM Grafted Nanoparticles

A dispersion of SiO_2 particles (0.49 grams (g) of 2327, 20 nm ammonium stabilized colloidal silica sol, 41% solids; Nalco, Naperville, IL) was placed in a 5 mL vial. The dispersion was diluted with 0.2 g of deionized water and 0.5 g of DMSO. To the stirred dispersion was added 33 mg of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea in 2 g of DMSO. After the addition, the dispersion was placed in an ultrasonic bath at 40°C for 2 hours. The vial was then capped and placed in an oven at 80°C for 24 hours. The resulting dispersion was analyzed by liquid chromatography. The spectrum showed a broad peak

with different retention time compared to that of the starting IRM silane. The dispersion was centrifuged to remove the solvents.

Example 9: Preparation of IRM Grafted Nanoparticles

A dispersion of SiO_2 particles (0.49 g of 2327, 20 nm ammonium stabilized colloidal silica sol, 41% solids; Nalco, Naperville, IL) was placed in a 5 mL vial. The dispersion was diluted with 0.2 g of deionized water and 0.5 g of DMSO. To the stirred dispersion was added 33 mg of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea in 2 g of DMSO. After the addition, the dispersion was placed in an ultrasonic bath at 40°C for 2 hours. The vial was then capped and placed in an oven at 80°C for 24 hours. The vial was cooled to room temperature and to the vial was added PEG triethoxysilane (12.4 mg, 0.0248 mmol available from GELEST, INC., Morrisville, PA). After the addition, the vial was capped and placed in an ultrasonic bath for 2 hours. The vial was then placed in an oven at 80°C for 24 hours. The dispersion was then centrifuged to remove the solvents.

Example 10: Preparation of IRM Grafted Nanoparticles

The procedure of Example 9 was repeated except that the amount of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea was reduced from 33 mg to 17 mg.

Example 11: Preparation of IRM Grafted Nanoparticles

The procedure of Example 9 was repeated except that the amount of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea was reduced from 33 mg to 8.5 mg.

Example 12: Preparation of IRM Grafted Nanoparticles

The procedure of Example 9 was repeated except that the amount of PEG triethoxysilane was increased from 12.4 mg to 31.0 mg.

Example 13: Preparation of IRM Grafted Nanoparticles

The procedure of Example 8 was repeated except that 34 mg of *N*-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea was used in lieu of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea.

Example 14: Preparation of IRM Grafted Nanoparticles

The procedure of Example 9 was repeated except that 34 mg of *N*-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea was used in lieu of *N*-[3-(4-amino-2-ethoxymethyl-6,7-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propyl]-*N'*-[3-(triethoxysilyl)propyl]urea.

Example 15: Preparation of IRM Grafted Nanoparticles

The procedure of Example 14 was repeated except that the amount of *N*-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea was reduced from 34 mg to 17 mg.

Example 16: Preparation of IRM Grafted Nanoparticles

The procedure of Example 14 was repeated except that the amount of *N*-[4-(4-amino-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]-*N'*-[3-(triethoxysilyl)propyl]urea was reduced from 34 mg to 8.5 mg.

Example 17: Preparation of IRM Grafted Nanoparticles

The procedure of Example 14 was repeated except that the amount of PEG triethoxysilane was increased from 12.4 mg to 31.0 mg.

Test Data

The beads prepared in Example 5 were tested for their ability to induce cytokines in the following manner. Twenty microliters (20 μL) of a slurry of the beads (80 mg beads/mL PBS) was added to 250 μL of human peripheral blood mononuclear cells (5 x 10^5 cells) in RPMI complete media and incubated overnight. 1:1 dilution duplicates were assayed for IFNα and TNFα concentrations by ELISA. The results are shown in the table

below where IFN and TNF are reported in picograms/mL and sd is the standard deviation. Control DYNABEADS are beads that were treated with buffer alone.

	IFNα (1)	IFNα (2)	Ave IFNα	sd		TNFα (1)	TNFα (2)	Ave TNFα	sd
IRM on DYNABEADS	1148.7	888.6	1018.7	130.0		33.2	45.8	39.5	6.3
Control DYNABEADS	5.7	1.4	3.5	2.1		26.1	17.2	21.7	4.5

The particles of Examples 1, 2, and 8-17 were tested in a single experiment using the method described above and did not induce significant amounts of either interferon alpha or tumor necrosis factor alpha at the concentrations tested.

The complete disclosures of the patents, patent documents and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control. Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.